

AMENDMENTS TO THE SPECIFICATION:

Please amend the paragraph beginning at page 4, line 14, as follows:

Figures 1A-1F. Letal is a new NKG2D ligand exhibiting a cytoplasmic domain.

Figs. 1A, The genomic sequence of Letal reveals the presence of 4 exons separated by 3 short introns, the sequences of the 4 exons being set forth in Fig. 1E (SEQ ID NOs:1-4) and a depiction of the translation of the cDNA into the amino acid sequence being set forth in Fig. 1F (SEQ ID NO:8). Fig. 1B, Alignment of Letal (SEQ ID NO:8) and ULBPs (SEQ ID NOs:5-7).

The transmembrane segment, spanning amino acids from 226 to 248, is marked with asterisks.

Fig. 1C, Phylogeny of all the murine and human NKG2D ligands so far characterized, generated with the topological algorithm of ClustalW. Fig. 1D, Letal is a ligand for NKG2D. All the results shown are representative of at least 3 experiments. Left: *Letal*-transduced K562 cells exhibit a much stronger staining by a polyclonal anti-Letal Ab than mock-transductants.

Incubation with GPI-specific Phospholipase C results in slight increase in binding, suggesting a positive effect in exposing epitopes, and confirming the transmembrane structure of Letal.

Shaded: *Letal*⁺ transductants; open thick: *Mock*-transductants; dotted: Treatment with Phospholipase C. Center, right: Control or *Letal*-transduced K562 and SKOV3 cells were incubated with a NKG2D-Fc protein, and stained with anti-human Ig mAb. Shaded: *Letal*⁺ transductants; open thick: *Mock*-transductants. When *Letal*⁺-K562 cells are treated with GPI-specific Phospholipase C, sNKG2D binding decreases, but it is still stronger than that of mock transductants, corresponding to the abundant expression of GPI-anchored NKG2D ligands in K562 cell line (dotted). No significant decrease is observed with SKOV3.

Please amend the paragraph beginning at page 7, line 23, as follows:

Figures 7A-7D. Letal and GLPD1 expression in normal and neoplastic ovarian tissues.

Fig. 7A, Quantification of Letal mRNA levels by TaqMan in human normal ovaries and benign tumors (n=6); borderline tumors (n=4); stage I (n=9), and stage III ovarian carcinomas (n=29).

Fig. 7B, Letal mRNA expression analyzed by ~~TaqMan~~ TAQMAN (real-time polymerase chain

reaction) PCR in tumor islets isolated by laser capture microdissection. 12 specimens were evaluated with CD3⁺ cells infiltrating tumor islets and 7 with no T-cells in tumor islets. Fig. 7C,

Kaplan-Meier curves for the duration of overall survival, according to the presence or absence of Letal mRNA in 38 patients with stage III epithelial ovarian cancer. Letal expression was

analyzed by Real-Time PCR. P values were derived with the use of log-rank statistic. Fig. 7D,

Quantification of GLPD1 mRNA levels by ~~TaqMan~~ TAQMAN (real-time polymerase chain

reaction) in the same specimens. Results are expressed as number of copies of the gene of interest per each 10⁶ GAPDH copies.

Please delete the paragraph beginning at page 25, line 6, in its entirety, and insert the following paragraph in lieu thereof:

Identification and characterization of the genomic and cDNA sequences of Letal. The amino acid sequence of the α -1 and α -2 domains of all the known human ligands for the NKG2D receptor (GenBank accession numbers: XM_015542, XM_027342, XM-015533, XM_044229, and XM_029639) were aligned in order to create patterns with the amino acids conserved in at least four of the five sequences and coded by a single exon. Genomic sequences at chromosome 6q25 were translated into the 6 possible open reading frames by using the ORF Finder Program (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) and were scanned for the presence of these

patterns with the PattinProt software (<http://pbil.ibcp.fr/>) (Combet et al, Trends Biochem. Sci. 25(3):147-150 (2000)). Based on these criteria, a sequence was focussed upon that was designated Letal. To search for 5'- and 3'-sequences of the novel gene, the amino acid sequences of ULBP1, ULBP2, and ULBP3 were used to perform a scan algorithm for the detection of genes by using FGENESH+ (<http://genomic.sanger.ac.uk/gf/gfs.shtml>) (Solovyev et al, Nucleic Acids Res. 27(1):248-250 (1999)) and GeneBuilder (<http://l25.itba.mi.cnr.it/~webgene/genebuilder.html>) (Milanesi et al, 15(7-8):612-621 (1999)). The primers Letal.F: 5'-CCATACCAGTGAGGGTGAATG-3' (SEQ ID NO:11) and Letal.R: 5'-CCCATGATTCACCTCTCTTGAG-3' (SEQ ID NO:12) were used to amplify by PCR the complete open reading frame for the predicted gene from the ovarian carcinoma cell line A2008. The putative cleavage sites of the prepropeptide were predicted with SignalP V2.0 (<http://www.cbs.dtu.dk/services/SignalP-2.0>) (Nielsen et al, Protein Eng. 10(1):1-6 (1997)) and the transmembrane domain with PRED-TMR software (<http://o2.db.uoa.gr/PRED-TMR/>) (Pasquier et al, Protein Eng. 12(5):381-386 (1999)). Clustalw (<http://www.ebi.ac.uk/clustalw>) was used to perform alignments and build the phylogenetic tree and PredictProtein (<http://maple.bioc.columbia.edu/pp/>) (Rost and Sander, J. Mol. Biol. 232(2):584-599 (1993)) was used to predict the secondary structure.

Please amend the paragraph beginning at page 31, line 27, as follows:

Using real-time quantitative PCR (~~TaqMan~~) (TAQMAN) (real-time polymerase chain reaction), abundant Letal transcripts were detected in normal small intestine by, and at lower levels in normal brain, breast, colon, spleen, skeletal muscle, uterus, thymus, placenta, blood lymphocytes and ovary (Figure 2A). RT-PCR analysis indicated the presence of Letal mRNA in

most colon cancer cell lines tested, but only in two out of fifteen ovarian carcinoma cell lines (Figure 2B). A shorter splicing variant encoding for a protein lacking 36 amino acids from the α -1 domain, corresponding to the GenBank Entry: AY054974, was found in 4 cancer cell lines. However, Letal was invariably the predominant form. No expression in immature dendritic cells was detected. Basal Letal mRNA levels increased 1.6-fold and 3-fold in A2008 cells upon infection with Herpes simplex virus or addition of TNF- α , respectively, whereas other inflammatory cytokines, hypoxia or starvation had little to no effect on Letal expression (Figure 3A). Human MICA/B and mouse RAE-1 family members are upregulated by 48-72 hr treatment with retinoic acid (RA) (Cerwenka et al, Immunity 12(6):721-727 (2000), Jinushi et al, Int. J. Cancer 104(3):354-361 (2003)). Surprisingly, a progressive decrease of Letal mRNA expression was found after treatment of A2008 cells with RA, which was maximum (6-fold) after 72 hr stimulation, suggesting that signals inducing transcriptional activation of NKG2D ligands are markedly different for each molecule (Figure 3B).

Please amend the paragraph beginning at page 42, line 1, as follows:

T-cells infiltrate tumor islets (intratumoral T-cells) in approximately 55% of ovarian cancers, while T-cells are exclusively detected in peritumoral stroma in the remainder (Zhang et al, N. Engl. J. Med. 348:203-213 (2003)). Simultaneous stimulation of the T-cell receptor and Letal induces proliferation of cytotoxic lymphocytes *in vitro* (Conejo-Garcia et al, Cancer Biol. Ther. 2 available online). To investigate whether Letal plays any role in the expansion of intratumoral T-cells *in vivo*, Letal levels in tumor islets showing intratumoral T cells and tumor islets lacking intratumoral T-cells were measured, using laser capture microdissection to procure highly pure samples of tumor islets. ~~TaqMan~~ TAQMAN (real-time polymerase chain reaction)

analysis of 19 different stage III specimens revealed a 30-fold higher Letal mRNA expression in islets infiltrated by T-cells compared to islets lacking T-cells ($P=0.041$; Figure 7B). Since most ovarian tumors express MHC-I by immunohistochemistry (Kooi et al, Cell Immunol. 174:116-128 (1996)) and flow cytometry, these data suggest that Letal may be involved in the enrichment of T-cells in tumor islets.